FORM PTO-1390

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE
TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 98 BA INS SAM

U.S. APPLN. NO. (If known, see 37 CFR 1.5) 09/830188

INTERNATIONAL APPLICATION NO.:	INTERNATIONAL FILING DATE:	PRIORITY DATE CLAIMED:
PCT/EP99/08031	22 October 1999	23 October 1998

TITLE OF INVENTION: CHELATING AGENTS FOR RADIOIMMUNOTHERAPY

APPLICANT(S) FOR DO/EO/US: Jean-François GESTIN, Anthony LOUSSOUARN and Alain FAIVRE-CHAUVET

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau. (see attached copy of PCT/IB/308)
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Item 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A **FIRST** preliminary amendment.
    [ ] A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:

International Preliminary Examination Report (PCT/IPEA/409)
International Search Report (PCT/ISA/210)
Abstract of the Disclosure on a separate sheet
Application Data Sheet

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 09/830188	INTERNATIONAL APPLICATION NO. PCT/EP99/08031	ATTORNEY'S DOCKET NO. 98 BA INS SAM

	CALCULATIONS PTO USE ONLY
17. [X] The following fees are submitted:	
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):	
Neither international preliminary examination fee (37 CFR1.482) nor international search fee (37 CFR1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... $ 1,000.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... $ 860.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... $ 710.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... $ 690.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... $ 100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00
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CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	18 - 20 =	0	X $18.00	$
Independent claims	1 - 3 =	0	X $80.00	$
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TOTAL OF ABOVE CALCULATIONS =	$ 990.00
Reduction of ½ for filing by small entity, if applicable. Applicant claims Small Entity Status under 37 CFR 1.27. +	$
SUBTOTAL =	$ 990.00
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a. [X] A check in the amount of $ **990.00** to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. **25-0120** in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required by 37 CFR 1.16 and 1.17, or credit any overpayment to Deposit Account No. **25-0120**. A duplicate copy of this sheet is enclosed.

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April 23, 2001

By Benoît Castel
Benoît Castel
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PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Jean-Francois GESTIN et al.

Serial No. 09/830,188 GROUP Unassigned

Filed April 23, 2001 Examiner Unassigned

CHELATING AGENTS FOR RADIOIMMUNOTHERAPY

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

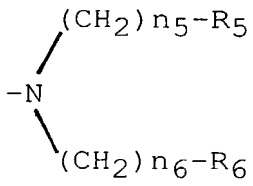
Sir:

The following amendments to the claims are submitted prior to examination of the above-referenced application.

IN THE CLAIMS:

Please amend the claims as follows:

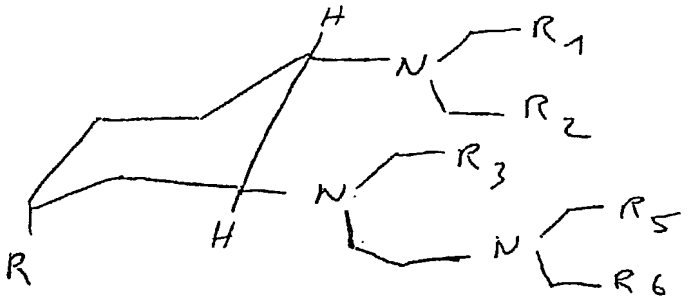
3. (amended) Compounds according to claim 1, characterized in that at least one, and more preferably two of R_1, R_2, R_3 and R_4, represent a group



4. (amended) Compounds according to claim 1, characterized in that R represents a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined in claim 1, and in particular R represents a group chosen among all the coupling functions for vector or solid support binding.

5. (amended) Compounds according to claim 1, characterized in that R represents a group carrying a function linked, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides.

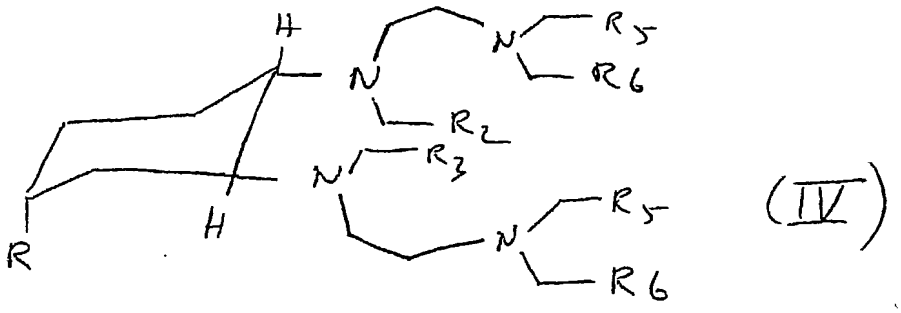
6. (amended) Compounds of the following formula (III) :



(III)

in which R_1, R_2, R_3, R_5 and R_6 independently from each other represent -COOH or $-PO(OH)_2$, and R is a group as defined in claim 2.

8. (amended) Compounds of the following formula (IV) :



(IV)

wherein R_2, R_5 and R_6, independently form each other, represent -COOH or $-PO(OH)_2$, and R is a group as defined in claim 2.

10. (amended) Complexes between a compound according to claim 1, and a radioactive element.

13. (amended) Use of a complex according to claim 11, for the manufacture of a medicament for radioimmunotherapy, such as for the treatment of cancers,

and more particularly for the treatment against metastase proliferation.

14. (amended) Pharmaceutical compositions characterized in that they comprise an effective amount of a complex according to claim 1, in association with a suitable pharmaceutical carrier.

16. (amended) Complexes according to claim 15, characterized in that the compound is chosen among those compounds wherein the group R comprises:

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,

- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound (in a first step of the method of diagnosis) to epitopes on the surface of specific cells in the organism,

- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

17. (amended) Use of a complex according to claim 15, for carrying out diagnosis methods such as radioimmunoscintigraphy.

18. (amended) Use of a compound of formula (I) as defined in claim 1, included compounds CDTPA and CTTHA, for:

- the manufacture of a medicament useful as antalgic, or for the treatment of pathologies where ionic imbalances occur, or against the formation of stones in the organism,

- carrying out a process for the detoxication of polluted medium, such as liquid phases polluted by bivalent or trivalent metals radioactives or not,

- carrying out a process for the radionuclides purification, said compound being bound to a solid phase,

- carrying out a bone scintigraphy, in particular in the frame of the diagnosis of osteoarticular pathology, particularly in bone cancer extension balance.

Respectfully submitted,

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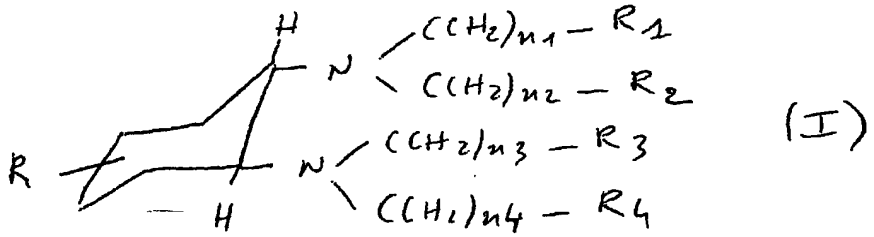
May 31, 2001

"MARKED-UP VERSION SHOWING CHANGES MADE"

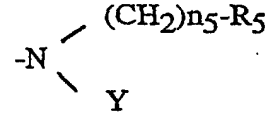
CLAIMS

1. Compounds of the following formula (I) :



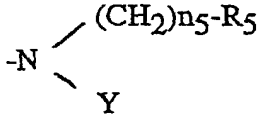
(I)

in which :

- n_1, n_2, n_3 and n_4, independently from each other, represent an integer from 1 to 5, preferably from 1 to 3,
- R_1, R_2, R_3 and R_4, independently from each other, represent :
  - . –COOH,
  - . $-PO(OH)_2$,
  - . 

wherein n_5 represents an integer from 1 to 5, preferably from 1 to 3, R_5 represents –COOH or $-PO(OH)_2$, and Y represents H or a group $-(CH2)n_6-R_6$ in which n_6 represents an integer from 1 to 5, preferably from 1 to 3, and R_6 represents –COOH or $-PO(OH)_2$,

provided that at least one of R_1, R_2, R_3 or R_4 represents a group

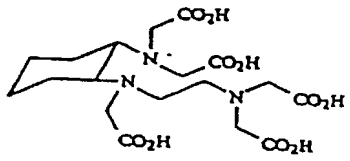


such as defined above,

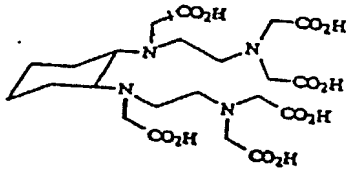
- R represents :
  - . H, or $-NHCOCH_3$, or
  - . a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, which are able to bind specifically with epitopes located at the surface of the cells of the organism, or to chemical or biological compounds located at the surface of a solid carrier, or

: a group carrying a function linked, if necessary via a binding site, to molecules as defined above,

the two following compounds, CDTPA and CTTHA, being excluded :



CDTPA

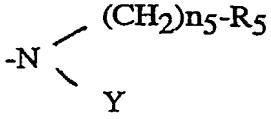


CTTHA

2. Compounds according to claim 1, characterized in that :

- when R_1, R_2, R_3 or R_4 represents -COOH or $-PO(OH)_2$, then n_1, n_2, n_3 or n_4 represents 1 respectively,

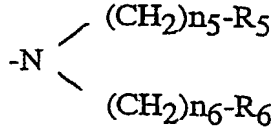
- when R_1, R_2, R_3 or R_4 represents a group



then n_1, n_2, n_3 or n_4 represents 2 or 3 respectively, and preferably 2,

- n_5, and optionally n_6, represents 1.

3. Compounds according to [claims 1 or 2], characterized in that at least one, and more preferably two of R_1, R_2, R_3 and R_4, represent a group



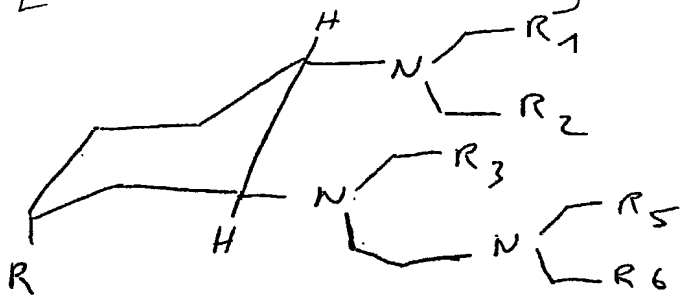
[wherein n_5, n_6, R_5 and R_6 are defined in claims 1 or 2].

4. Compounds according to [anyone of claims 1 to 3], characterized in that R represents a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined in

claim 1, and in particular R represents a group chosen among all the coupling functions for vector or solid support binding.

5. Compounds according to anyone of claims 1 to 3, characterized in that R represents a group carrying a function linked, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined in claim 1.

6. Compounds according to anyone of claims 1 to 5 of the following formula (III) :



(III)

in which R_1, R_2, R_3, R_5 and R_6 independently from each other represent -COOH or -$PO(OH)_2$, and R is a group as defined in claims 2 to 5.

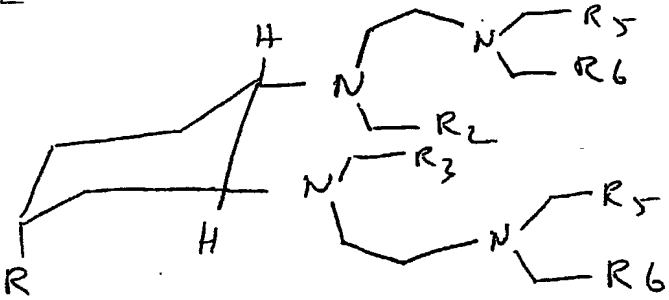
7. Compounds according to claim 6, of formula (III) wherein :

. $R_1 = R_5 = R_6 = COOH$ and $R_2, = R_3 = PO(HO)_2$, or

. $R_1 = R_2 = R_3 = R_5 = R_6 = COOH$, or

. $R_1 = R_2 = R_3 = R_5 = R_6 = PO(OH)_2$.

8. Compounds according to anyone of claims 1 to 5, of the following formula (IV) :



(IV)

wherein R_2, R_5 and R_6, independently form each other, represent -COOH or -$PO(OH)_2$, and R is a group as defined in claims 2 to 5.

9. Compounds according to claim 8 of formula (IV) wherein :

. $R_2 = R_3 = PO(OH)_2$, and $R_5 = R_6 = COOH$, or

. $R_2 = R_3 = R_5 = R_6 = COOH$, or

. $R_2 = R_3 = R_5 = R_6 = PO(OH)_2$.

10. Complexes between a compound according to anyone of claims 1 to 9, and a radioactive element.

11. Complexes according to claim 10, characterized in that said radioelements are α or β emitter radiometals.

12. Complexes according to claim 11, characterized in that the compound is chosen among those defined in anyone of claims 5 to 9, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,
- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound, in a first step of the treatment, to epitopes on the surface of specific cells in the organism,
- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

13. Use of a complex according to claims 11 or 12, for the manufacture of a medicament for radioimmunotherapy, such as for the treatment of cancers, and more particularly for the treatment against metastase proliferation.

14. Pharmaceutical compositions characterized in that they comprise an effective amount of a complex according to claims 11 or 12, in association with a suitable pharmaceutical carrier.

15. Complexes according to claim 10, characterized in that the radioelements are γ emitter radiometals.

16. Complexes according to claim 15, characterized in that the compound is chosen among those defined in anyone of claims 5 to 9, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,

- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound (in a first step of the method of diagnosis) to epitopes on the surface of specific cells in the organism,

- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

17. Use of a complex according to claims 15 or 16, for carrying out diagnosis methods such as radioimmunoscintigraphy.

18. Use of a compound of formula (I) as defined in claim 1 to 9, included compounds CDTPA and CTTHA, for :

- the manufacture of a medicament useful as antalgic, or for the treatment of pathologies where ionic imbalances occur, or against the formation of stones in the organism,

- carrying out a process for the detoxication of polluted medium, such as liquid phases polluted by bivalent or trivalent metals radioactives or not,

- carrying out a process for the radionuclides purification, said compound being bound to a solid phase,

- carrying out a bone scintigraphy, in particular in the frame of the diagnosis of osteoarticular pathology, particularly in bone cancer extension balance.

PCT

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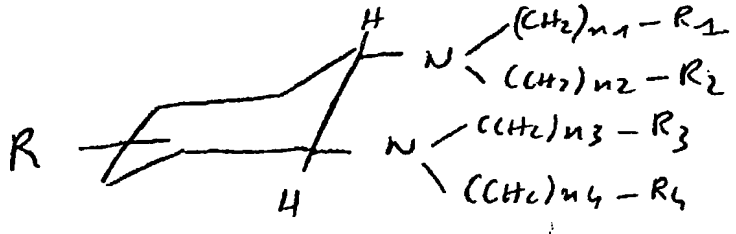
(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

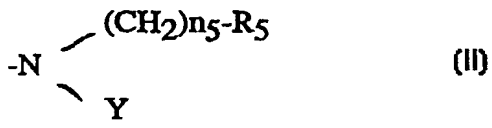
(54) Title: CHELATING AGENTS FOR RADIOIMMUNOTHERAPY

(57) Abstract

The invention relates to compounds of formula (I): in which: n_1, n_2, n_3 and n_4, represent an integer from 1 to 5, R_1, R_2, R_3 and R_4, independently from each other, represent –COOH, $-PO(OH)_2$, at least one of R_1, R_2, R_3 or R_4 represents a group (II), wherein n_5 represents an integer from 1 to 5, R_5 represents –COOH or $-PO(OH)_2$, and Y represents H or a group $-(CH2)n_6-R_6$ in which n_6 represents an integer from 1 to 5, and R_6 represents –COOH or $-PO(OH)_2$, R represents H, or a group carrying a function linked to molecules able to bind with epitopes at the surface of cells. The invention also relates to the processes of preparation of said compounds, and to their use in pharmaceutical compositions and in diagnosis methods.



(I)



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CHELATING AGENTS FOR RADIOIMMUNOTHERAPY

The invention relates to compounds useful as chelating agents, complexes between said compounds and radioelements, and to their uses, in particular in pharmaceutical compositions and compositions for the diagnosis of pathologies such as cancers.

Immunotherapy with radiolabeled antibodies should allow fairly specific targeting of certain cancers (Schubiger et al., 1996; Parker, 1990). However, iodine-131 (Bardies et al., 1992; Stein et al., 1995) may not be the best isotope for tumor therapy because of its limited specific activity, low beta-energy, relatively long half-life and strong gamma emission.

Another approach to improving therapeutic efficacy is the use of replacement isotopes with better physical properties. Chelators that can hold radiometals with high stability under physiological conditions are essential to avoid excessive radiation damage to non-target cells. Moreover, the development of new bifunctional chelating agents is essential for this purpose. Thus synthesis of new chelating agents able to bind radiometals such as rhenium-188, yttrium-90, samarium-153 or Bismuth-213 and in general all the α and β particles emitters will be required to possess sufficiently stable chelators.

Accordingly, one of the aim of the invention is to provide chelating agents forming stable complexes *in vivo* with the numerous potential candidates for such applications.

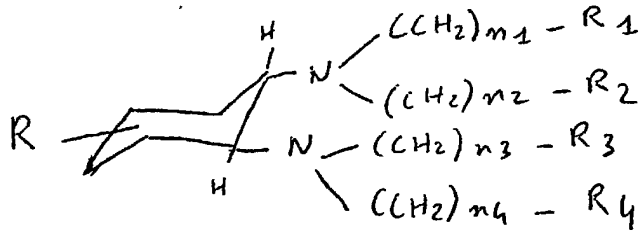
The stability of a non-macrocyclic ligand can be favourably influenced by the preorganization of the open chain. In fact, a semi-rigid structure such as that of *trans* 1-2 diaminocyclohexane limits the rotation of the ethylene bridge, so that the purpose of the cyclohexane design is to preorient the four pendent arms in a skew position.

A first investigation (Mease et al., 1990), which was guided by a study performed on polyaminocarboxylic acid ligands incorporating the skeleton of ethylenediaminetetraacetic acid (EDTA) in a cyclohexane structure, showed the influence of this semi-rigid structure on the stability of the resulting complexes. A second study (Goeckeler et al., 1987) of the stability of lanthanides as ^{153}Sm-polyaminophosphonic acid complexes showed that ethylenediamine tetramethylphosphonic acid (EDTMP) derivatives allow stable quantitative ^{153}Sm chelation.

The (1*R**, 2*R**, 4*S**)-4-acetamido-1,2-diaminocyclohexane dihydro chloride compound, the structural derivative of *trans*-1,2-diaminocyclohexane, have been prepared (Gestin et al., 1997; Loussouarn, et al., 1998). This intermediate, which is functionalized at position 4 of the cycle by a protected amine termination (Meares et al., 1984) for future covalent attachment to biomolecules, allows the introduction of different chelating groups via the free amines.

The Inventors have developed a new simple and efficient synthesis pathway from *trans* -1,2- diaminocyclohexane to provide access to a new class of semi-rigid chelating agents. This same reactional scheme applies to the reactional intermediary, (1*R**, 2*R**, 4*S**)-4-acetamido-1,2-diaminocyclohexane dihydrochloride, which allows the synthesis of these same chelating agents, though functionalized back of the cycle by a termination allowed coupling to an antibody or any other biological substance such as a hapten.

The present invention relates to compounds of the following formula (I) :



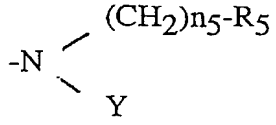
(I)

in which :

- n_1, n_2, n_3 and n_4, independently from each other, represent an integer from 1 to 5, preferably from 1 to 3,
- R_1, R_2, R_3 and R_4, independently from each other, represent :
  - . –COOH,
  - . $-PO(OH)_2$,
  - . $-N\begin{matrix}\diagup (CH_2)n_5\text{-}R_5 \\ \diagdown Y\end{matrix}$

wherein n_5 represents an integer from 1 to 5, preferably from 1 to 3, R_5 represents –COOH or $-PO(OH)_2$, and Y represents H or a group $-(CH2)n_6\text{-}R_6$ in which n_6 represents an integer from 1 to 5, preferably from 1 to 3, and R_6 represents –COOH or $-PO(OH)_2$,

provided that at least one of R_1, R_2, R_3 or R_4 represents a group



such as defined above,

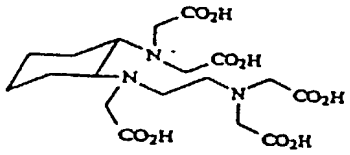
- R represents :

. H, or $-NHCOCH_3$, or

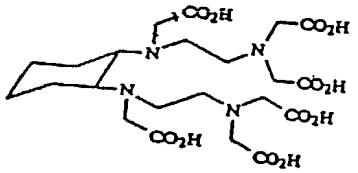
. a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, which are able to bind specifically with epitopes located at the surface of the cells of the organism, or to chemical or biological compounds located at the surface of a solid carrier, or

. a group carrying a function linked, if necessary via a binding site, to molecules as defined above,

the two following compounds, CDTPA and CTTHA, being excluded :



CDTPA

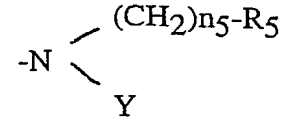


CTTHA

The invention relates more particularly to compounds of formula (I) such as defined above, characterized in that :

- when R_1, R_2, R_3 or R_4 represents -COOH or $-PO(OH)_2$, then n_1, n_2, n_3 or n_4 represents 1 respectively,

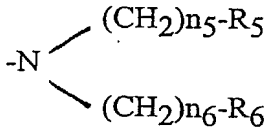
- when R_1, R_2, R_3 or R_4 represents a group



then n_1, n_2, n_3 or n_4 represents 2 or 3 respectively, and preferably 2,

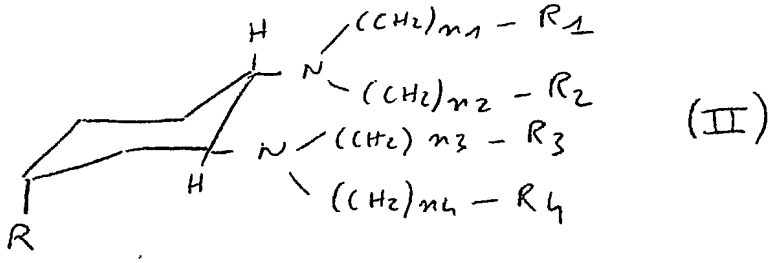
- n_5, and optionally n_6, represents 1.

The invention also relates more particularly to compounds of formula (I) such as defined above, characterized in that at least one, and more preferably two of R_1, R_2, R_3 and R_4, represent a group



wherein n_5, n_6, R_5 and R_6 are defined above.

Preferred compounds of formula (I) such as defined above, wherein R is different from hydrogen, are compounds of the following formula (II) :



(II)

wherein n_1, n_2, n_3, n_4, R_1, R_2, R_3, R_4 and R are such as defined above.

The invention relates more particularly to compounds of formula (I) or (II) such as defined above, characterized in that R represents a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined above, and in particular R represents a group chosen among all the coupling functions for vector or solid support binding, such as the following groups :

. alcohol group, such as -OH,

. amino group, such as $-NH_2$, $-NO_2$,

. aldehyde group, such as -CHO,

. carboxylic group, such as -COOH,

. anhydride group, such as -CO-O-CO-R",

. $-CO-CH_2X$, wherein X represents an halogen atom, such as Cl or Br,

. -CO-X, wherein X represents an halogen atom, such as Cl or Br,

. a diazonium ion N_2^+,

. an activated ester, such as -COOR", R" = ethyl or N-hydrosuccinimide,

. a sulfonic group, such as SO_3H,

. a thiocyanate group, such as -NCS, or an isocyanate -NCO, or a -NH-NCS group

. a thiol group, such as -SH,

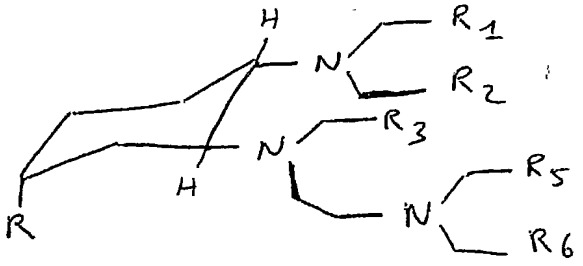
. a disulfure group, such as -S-S-R".

The invention also concerns compounds of formula (I) or (II) such as defined above, characterized in that R represents a group carrying a function linked, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined above, and more particularly R represents a group chosen among the following groups :

. -O-CO-R',
. -NH-CO-R',
. -NH-CS-R',
. -CH=N-R',
. -CO-NH-R',
. -CO-CH_2-NH-R',
. -N=N-NH-R',
. -SO_2-NH-R',
. -NH-CS-NH-R',
. -thioether-R',
. -CO-S-R',
. -CO-CH_2-S-R',
. -S-S-R',
. -NH-CH_2-R',
. -CO-NH-N=CH-R',
. -CS-NH-N=CH-R',

wherein R' represents said molecule.

The invention concerns more specifically compounds such as described above of the following formula (III) :



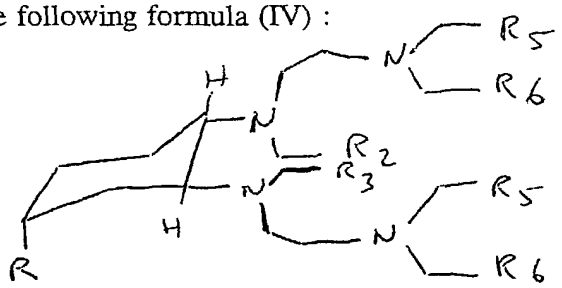
(III)

in which R_1, R_2, R_3, R_5 and R_6 independently from each other represent -COOH or -PO(OH)2, and R is a group as defined above.

Preferred compounds of formula (III) are such that :

. $R_1 = R_5 = R_6 = COOH$ and $R_2, = R_3 = PO(HO)_2$, or
. $R_1 = R_2 = R_3 = R_5 = R_6 = COOH$, or
. $R_1 = R_2 = R_3 = R_5 = R_6 = PO(OH)_2$.

The invention also concerns more specifically compounds such as described above, of the following formula (IV) :



(IV)

wherein R_2, R_5 and R_6, independently form each other, represent -COOH or -$PO(OH)_2$, and R is a group as defined above.

Preferred compounds of formula (IV) are such that :

. $R_2 = R_3 = PO(OH)_2$, and $R_5 = R_6 = COOH$, or

. $R_2 = R_3 = R_5 = R_6 = COOH$, or

. $R_2 = R_3 = R_5 = R_6 = PO(OH)_2$.

The invention also relates to complexes between a compound such as described above, and a radioactive element, said complexes resulting from the association of said radioelement with the -COOH and/or -$PO(OH)_2$ groups of said compound (the bonds between said radioelement and said compound being ionic bonds).

The above-mentioned radioelements are more particularly α, β or γ emitter radiometals, and preferably from the groups of actinides or lanthanides.

The invention relates more particularly to complexes such as described above, characterized in that said radioelements are α or β emitter radiometals (susceptible to be used in therapy, and more particularly in radioimmunotherapy in the frame of cancer treatments).

Advantageously, α emitter radiometals are chosen among the followings : Actinium 225, Bismuth 213.

Advantageously, β emitter radiometals are chosen among the followings : ^{33}P, ^{199}Au, ^{121}Sn, ^{177}Lu, ^{67}Cu, ^{105}Rh, ^{47}Sc, ^{77}As, ^{153}Sm, ^{159}Gd, ^{143}Pr, ^{186}Re, ^{111}Ag, ^{149}Pm, ^{109}Pd, ^{166}Ho, ^{32}P, ^{188}Re, ^{194}Ir, ^{142}Pr, ^{90}Y.

Preferred complexes with radiometals used in therapy, as defined above, are such that the compound is chosen among those wherein R represents a group carrying a function linked, if necessary via a binding site, to molecules as defined above, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,

- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound (in a first step of the treatment) to epitopes on the surface of specific cells in the organism,

- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

The invention also concerns the use of a complex such as described above, for the manufacture of a medicament for radioimmunotherapy (also called radiopharmaceutical), in particular for the treatment of cancers, or for the treatment against metastase proliferation.

More particularly, the invention relates to the use of a complex such as defined above, for the manufacture of a medicament for the treatment of :

- lung cancer, said complex preferably being such that it comprises a radioelement chosen among : ^{188}Re, ^{186}Re, ^{153}Sm, ^{67}Cu and ^{90}Y, and wherein R comprises an antibody specific for lung cancer cells, such as Anti N-CAM Antibody, Anti CEA Antibody, Anti Carbohydrates Antibodies, or an hapten chosen among Anti N-CAM-679 Bispecific antibody, Anti CEA-679 Bispecific antibody, Anti Carbohydrates-679 Bispecific antibody, Anti N-CAM-734 Bispecific antibody, Anti CEA-734 Bispecific antibody, Anti Carbohydrates-734 Bispecific antibody,

- liver and pancreatic cancers, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for liver and pancreatic cancer cells, such as antibodies and haptens described above in the case of lung cancer,

- ovarian cancer, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for ovarian cancer cells, such as OC125, MOV18, MOV19, OVTL3, or an hapten chosen among OC125-679 Bispecific antibody, MOV18-679 Bispecific antibody, MOV19-679 Bispecific antibody, OVTL3-679 Bispecific antibody, OC125-734 Bispecific antibody, MOV18-734 Bispecific antibody, MOV19-734 Bispecific antibody, OVTL3-734 Bispecific antibody,

- bladder cancer, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and

wherein R comprises an antibody specific for bladder cancer cells, such as AC48-127, or an hapten chosen among 48-127 Bispecific antibody, 48-127-679 Bispecific antibody, 48-127-734 Bispecific antibody,

- colorectal cancer, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for colorectal cancer cells, such as Anti CEA Antibody, Anti Carbohydrates Antibodies, or an hapten chosen among Anti CEA-679 Bispecific antibody, Anti Carbohydrates-679 Bispecific antibody, Anti CEA-734 Bispecific antibody, Anti Carbohydrates-734 Bispecific antibody,

- thyroïd medullary cancer, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for thyroïd medullary cancer cells, such as Anti CEA Antibody, or an hapten chosen among Anti CEA-679 Bispecific antibody, Anti CEA-734 Bispecific antibody,

- lymphoma, said complex preferably being such that it comprises a radioelement chosen among : ^{213}Bi, ^{225}Ac, ^{153}Sm, and ^{67}Cu, and wherein R comprises an antibody specific for lymphoma cells, such as specific antibody against expressed antigens surfaces lymphocyte cells, e.g. CD19, CD37, or an hapten such as bispecific antibody against expressed antigens surfaces lymphocyte cells, e.g. CD19-679, CD37-679, CD19-734, CD37-734,

- myeloma, said complex preferably being such that it comprises a radioelement chosen among : ^{213}Bi, ^{225}Ac, ^{153}Sm, and ^{67}Cu, and wherein R comprises an antibody specific for myeloma cells, such as specific antibody against expressed antigens surfaces myeloma cells, e.g. BB4, or an hapten such as bispecific antibody against expressed antigens surfaces myeloma cells, BB4-679, BB4-734,

- osteoarticular pathology, particularly in bone cancer extension balance.

The invention also concerns pharmaceutical compositions characterized in that they comprise an effective amount of a complex such as described above, in association with a suitable pharmaceutical carrier.

Pharmaceutical compositions according to the invention are more particularly characterized in that they are in a form suitable for an IV or IP administration in located areas.

Preferred pharmaceutical compositions according to the invention, are characterized in that the daily dosage is comprised between 1 and 100MBq /kg, e.g. between 3,7 and 74MBq/kg.

The invention also relates to complexes, as defined above, between a compound such as described above, and a radioactive element, characterized in that the radioelements are γ emitter radiometals (i.e. radiometals susceptible to be used in diagnosis methods, such as radioimmunoscintigraphy).

Advantageously, said radiometals arechosen among ^{111}In, ^{99m}Tc, ^{64}Cu.

Preferred complexes with radiometals used in diagnosis, as defined above, are such that the compound is chosen among those wherein R represents a group carrying a function linked, if necessary via a binding site, to molecules as defined above, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,
- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound (in a first step of the method of diagnosis) to epitopes on the surface of specific cells in the organism,
- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

The invention also concerns the use of a complex such as described above, for carrying out diagnosis methods such as radioimmunoscintigraphy.

More particularly, the invention concerns the use of a complex such as defined above, for carrying out the following diagnosis methods by radioimmunoscintigraphy :

- diagnosis of cancers, such as cited above, the complex used being preferably such that it comprises ^{111}In, or ^{99m}Tc as radioelements, and R comprises an antibody or a hapten specific for such cancer cells, such as antibodies or haptens mentioned above in the frame of the cancers listed above,
- diagnosis of cardiovascular diseases, such as graft rejection, myocardic infarcts,
- diagnosis of cerebral diseases,
- diagnosis of renal diseases, in paricular in the study of individual kidney functions, location of ectopic kidney, renal filtration and secretion troubles,
- vascular diseases, such as embolism and thrombosis, the complex used being preferably such that it comprises ^{111}In, or ^{99m}Tc as radioelements, and R comprises an antibody such as anti platelets or anti fibrin antibodies.

The invention also concerns the use of the complexes defined above for carrying out bone scintigraphy, in particular in the frame of the diagnosis of osteoarticular pathology, particularly in bone cancer extension balance.

The invention also relates to methods for the *in vitro* diagnosis of pathologies listed above, characterized in that it comprises the steps of incubating a biological sample, such as serum, plasma urines, with a complex as described above, the components of the biological sample being fixed to a solid carrier, rinsing the solid carrier and detecting the γ emission of the complex bound to the components of the sample on the solid carrier.

The invention also concerns the kits for carrying out said diagnosis methods, said kits comprising complexes as described above according to the invention.

The invention also relates to the use of a compound of formula (I) defined above, included compounds CDTPA and CTTHA, for the manufacture of a medicament for the treatment of pathologies where ionic imbalances occur, or against the formation of stones in the organism.

The invention also relates to the use of a compound of formula (I) defined above, included compounds CDTPA and CTTHA, for carrying out a process for the detoxication of polluted medium, such as liquid phases polluted by bivalent or trivalent metals radioactives or not.

The invention also concerns a process for the detoxication of a polluted medium comprising the steps of contacting said medium with a compound as defined above, advantageously itself bound to a solid carrier, and recovering said medium substantially free of contaminants which are bound to said compound on the solid carrier.

The invention also relates to the use of a compound of formula (I) defined above, included compounds CDTPA and CTTHA, for carrying out a process for the radionuclides purification, said compound of the invention being bound to a solid phase.

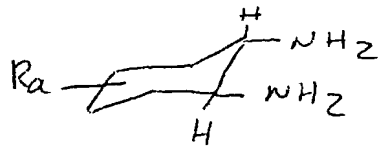
The invention also relates to the use of a complex between of formula (I) defined above, included compounds CDTPA and CTTHA, for carrying out a bone scintigraphy, in particular in the frame of the diagnosis of osteoarticular pathology, particularly in bone cancer extension balance.

The invention also relates to processes for preparing compounds and complexes as described above.

A process for the preparation of compounds according to the invention, comprises the following steps :

The invention also relates to processes for preparing compounds and complexes as described above. A process for the preparation of compounds according to the invention, comprises the following steps :

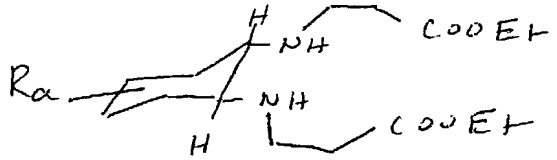
- contacting trans-1,2-diaminocyclohexane of the following formula a :



a

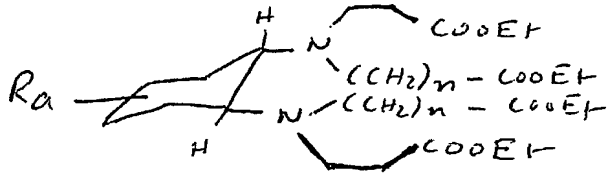
wherein R_a is H or $NHCOCH_3$,

* either with vinyl propionate, preferably by stirring 20h at room temperature, leading to the following compound b



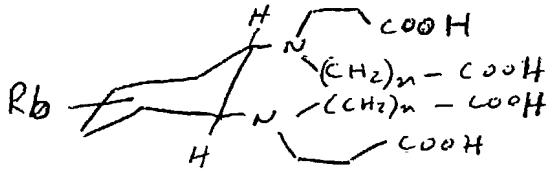
b

. contacting compound b with $X-(CH_2)_n-COOEt$, wherein X represents an halogen atom, and n represents an integer from 1 to 5, preferably at reflux during 15h, leading to the following compound c



c

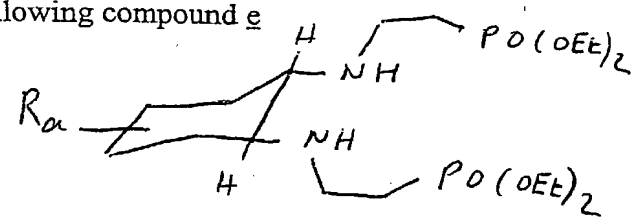
. treating compound c with HCl, preferably 6N HCl at reflux overnight, leading to the following compound d



d

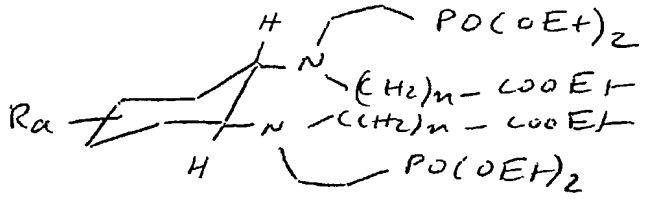
wherein R_b represents H or NH_2,

* or with diethyl vinyl phosphonate, preferably by stirring 15h at reflux, leading to the following compound e



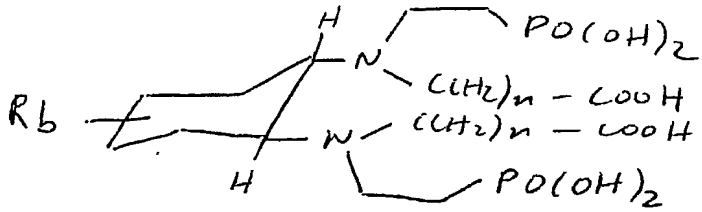
e

. contacting compound e with X-$(CH_2)_n$-COOEt, wherein X represents an halogen atom, and n represents an integer from 1 to 5, preferably at reflux during 15h, leading to the following compound f



f

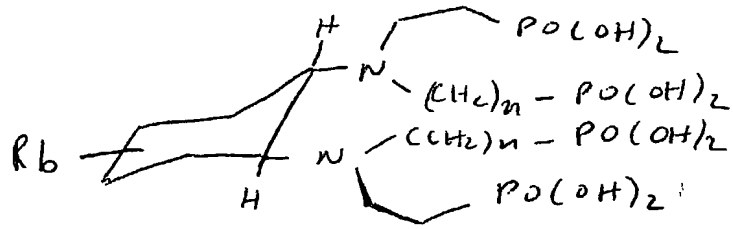
. treating compound f with HCl, preferably 6N HCl at reflux overnight, leading to the following compound g



g

wherein R_b represents H or NH_2,

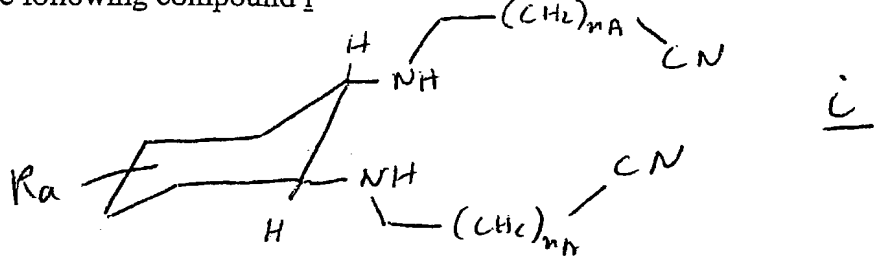
. if desired, treating compound g with phosphorous acid, preferably by stirring 30 mn at 80°C, leading to the following compound h



h

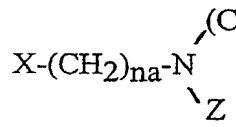
Another process for the preparation of compounds according to the invention, comprises the following steps :

- contacting the compound of formula a described above with a compound of formula H_2C=CH-$(CH_2)_{nA}$-CN wherein nA = 0 (acrylonitrile), or nA represents a integer from 1 to 3, preferably at room temperature during 20h, leading to the following compound i

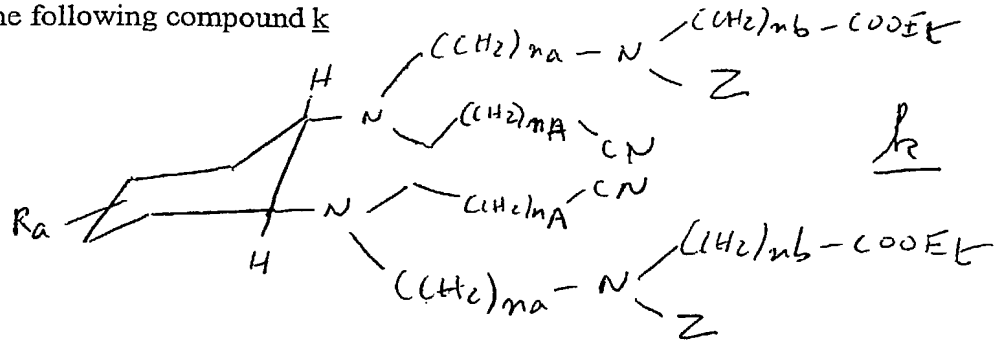


i

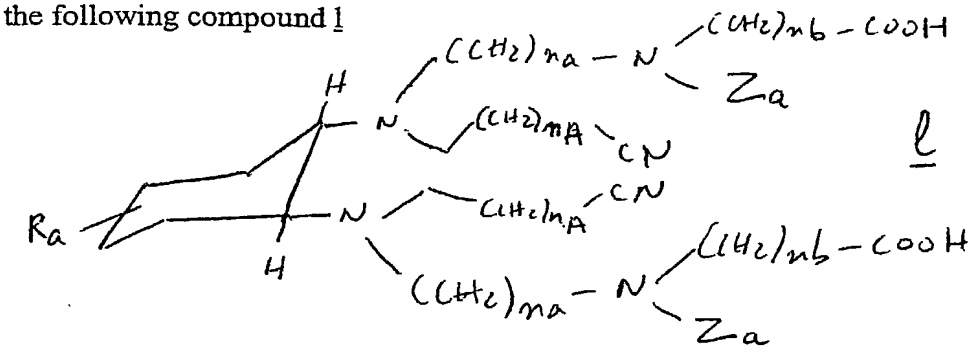
- contacting compound i with the following compound j



wherein X represents an halogen atom, na and nb, independently from each other represent an integer from 1 to 5, Z represents H or $(CH_2)_{nc}$-COOEt, and nc represents an integer from 1 to 5, preferably at 70°C during 2 days, leading to the following compound k



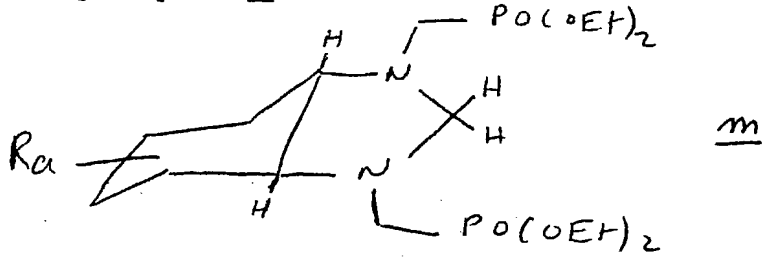
. treating compound k with HCl, preferably 6N HCl at reflux overnight, leading to the following compound l



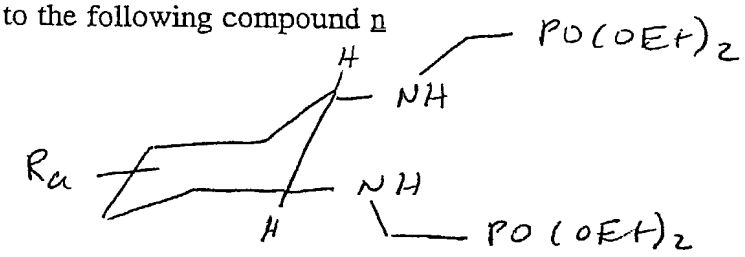
wherein Za represents H or -(CH2)nb-COOH, nA, na and nb being such as defined above, and R_b represents H or NH_2.

Another process for the preparation of compounds according to the invention, comprises the following steps :

- contacting the compound of formula a described above with paraformaldehyde and diethylphosphite, preferably in THF at reflux during 4h, leading to the following compound m



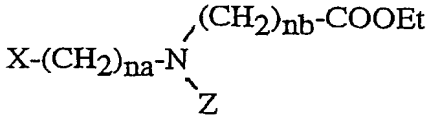
- treating compound m with HCl, preferably 3N HCl in MeOH at 50°C overnight, leading to the following compound n



m

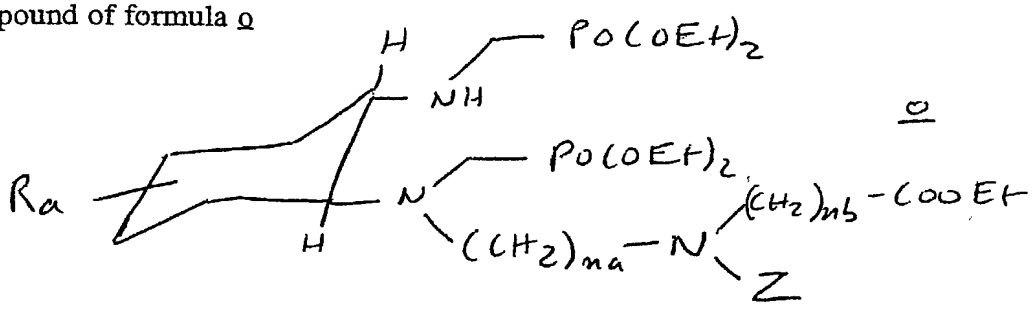
- contacting compound n :

* either with 1 equivalent of compound j



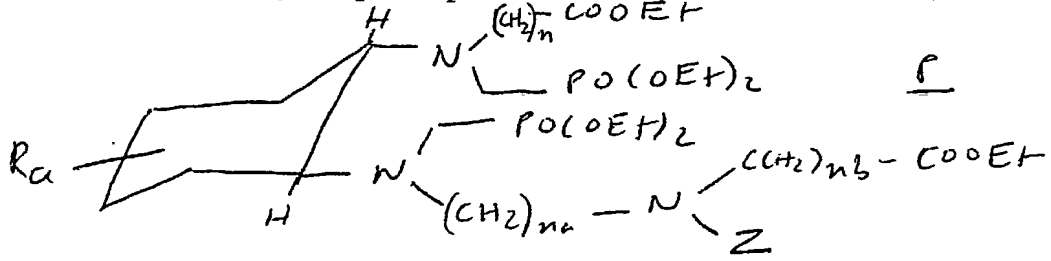
j

as described above, preferably at 70°C during 2 days, leading to the following compound of formula o



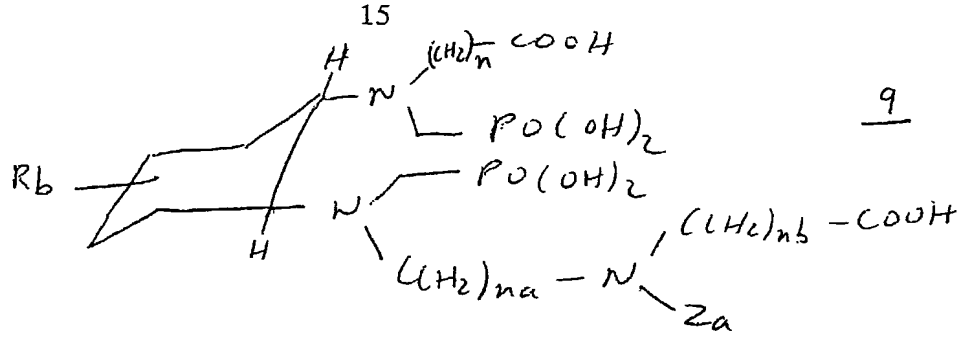
o

. contacting compound o with X-$(CH_2)_n$-COOEt, wherein X represents an halogen atom, and n represents an integer from 1 to 5, preferably at reflux during 15h, leading to the following compound p



p

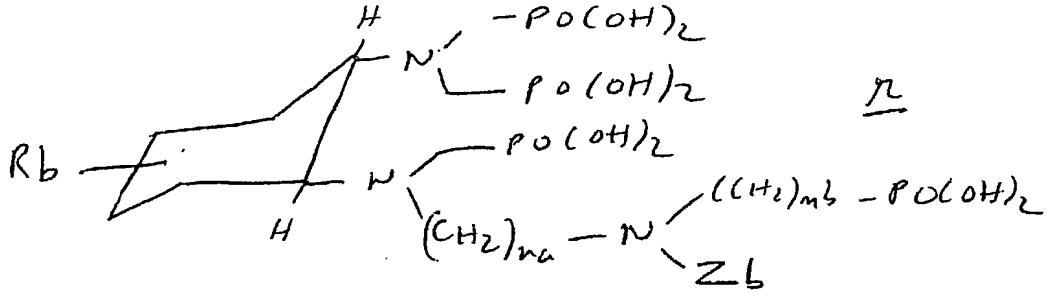
. treating compound p with HCl, preferably 6N HCl at reflux overnight, leading to the following compound q



q

wherein Za represents H or -$(CH_2)_{nb}$-COOH, na, nb and n being such as defined above, R_b represents H or NH_2,

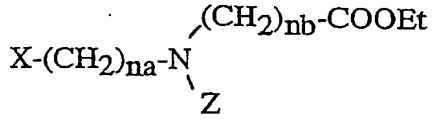
. if desired, treating compound q with phosphorous acid, preferably by stirring 30 mn at 80°C, leading to the following compound r



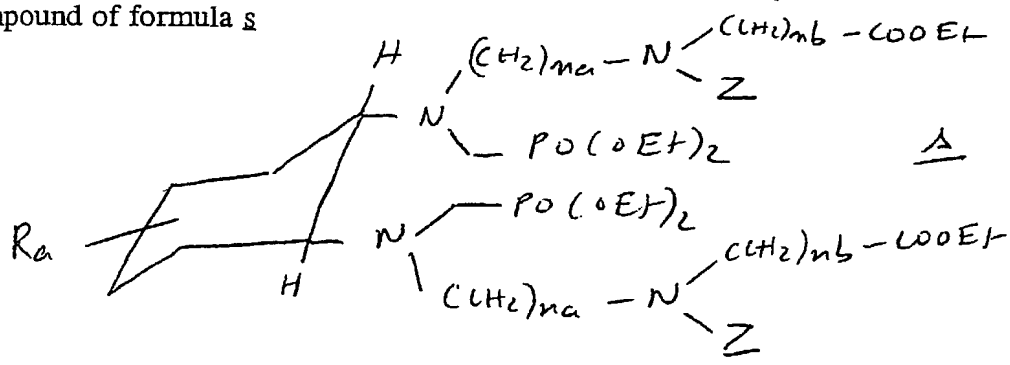
r

wherein Zb represents H or -$(CH_2)_{nb}$-$PO(OH)_2$

* or with 2 equivalents of compound j

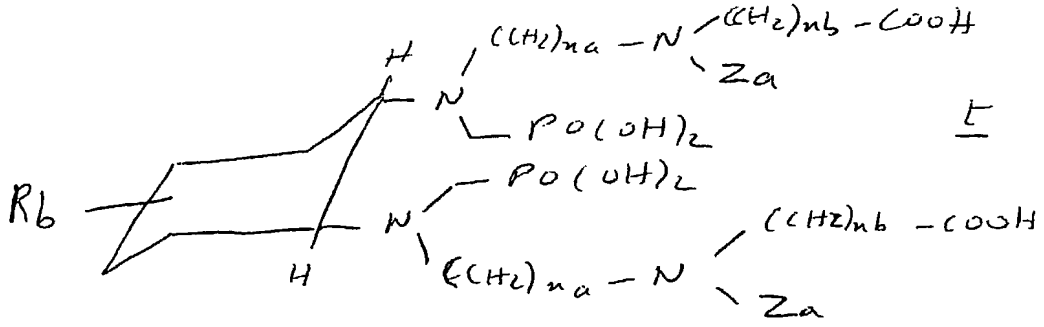


as described above, preferably at 70°C during 2 days, leading to the following compound of formula s



s

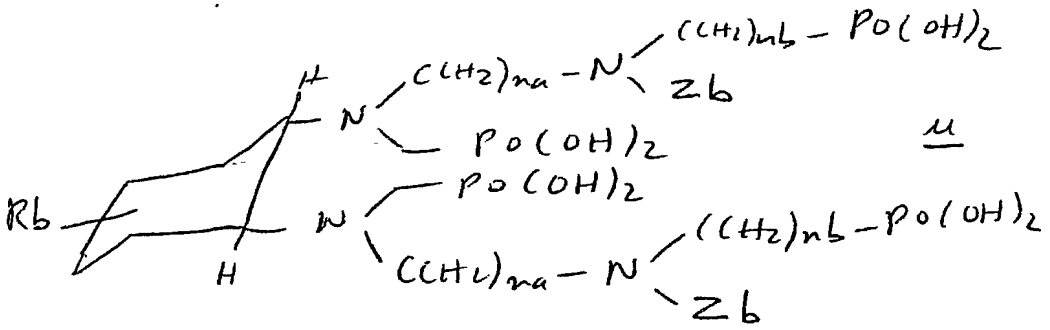
. treating compound s with HCl, preferably 6N HCl at reflux overnight, leading to the following compound t



t

wherein Za represents H or -$(CH_2)_{nb}$-COOH, na, nb and n being such as defined above, R_b represents H or NH_2,

. if desired, treating compound t with phosphorous acid, preferably by stirring 30 mn at 80°C, leading to the following compound u



u

wherein Zb represents H or -$(CH_2)_{nb}$-$PO(OH)_2$.

Compound of formula a can be obtained according to the method described in Gestin et al., 1997, and Loussouarn et al., 1998.

Compounds wherein Rb represents NH_2 obtained according to the processes described above, can then be transformed in order to correspond to compounds of formula (I) wherein R represents a group carrying a function liable to bind, if necessary via a binding site, to molecules as defined above.

By way of example, compounds of formula (I) wherein R represents -N=C=S, can be obtained by treatment of said compounds wherein Rb represents NH_2 with CSCl2, preferably in acidic or basic conditions.

Compounds of formula (I) wherein R represents a group carrying a function linked, if necessary via a binding site, to molecules as defined above,

can then be obtained by coupling said compounds, wherein R represents a group carrying a function liable to bind to said molecules, with said molecules.

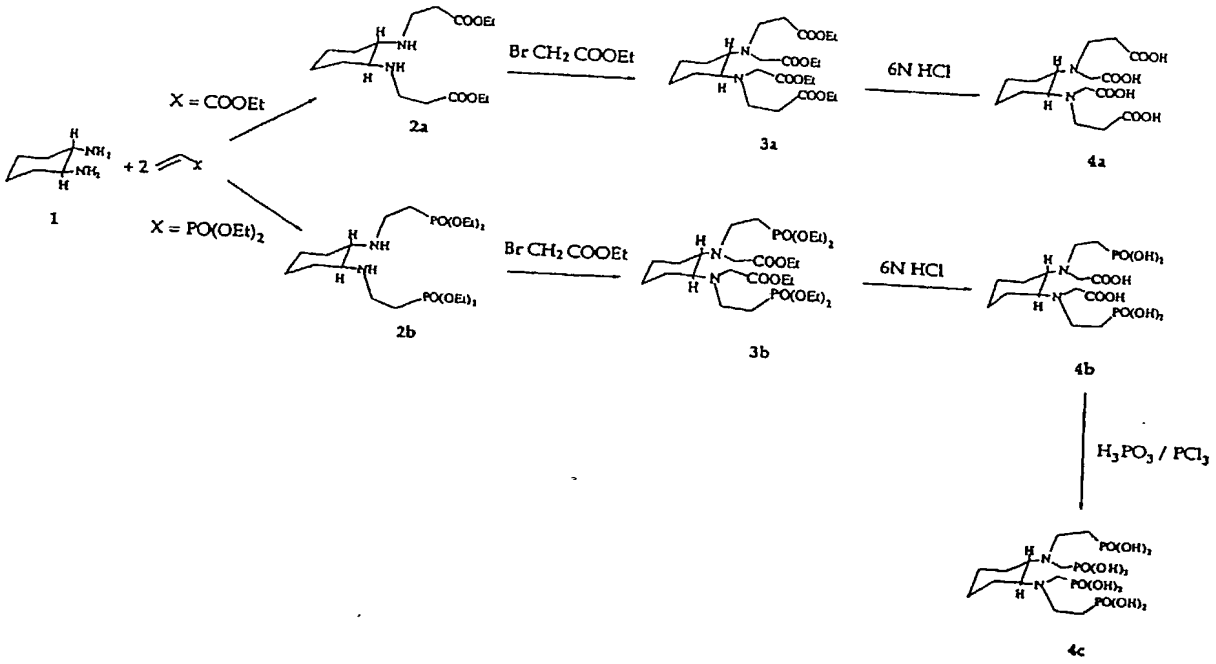
Complexes according to the invention are advantageously obtained by incubating the compounds withe the radioelements at 37°C during 3 hours.

The invention will be further illustrated in the following examples for the preparation of compounds and complexes according to the invention.

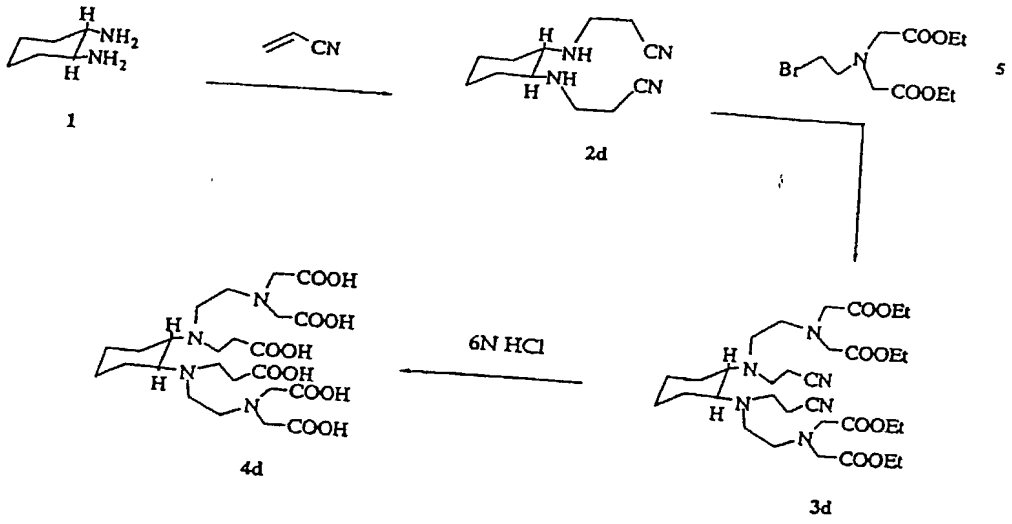
In order to save the functionalized reactional intermediate, i.e. the (1*R**, 2*R**, 4*S**)-4-acetamido-1,2-diaminocyclohexane dihydro chloride compound, the commercial product, *trans*-1,2- diaminocyclohexane **1**, has been used as starting material.

Two approach routes to differently substituted amines were carried out successfully.

- The first depicted in schemes I and II was the Michael type addition of primary amines to some vinylic derivatives to provide monoaddition with high selectivity (Bergeron et al., 1981), allowing N-alkylation to be envisaged at this step. In strategy depicted in scheme I, compounds **2a** and **2b** were alkylated by ethyl bromoacetate under conditions recommanded by Studer (Studer and Meares, 1992) (KI and Na_2CO_3) to give tetraesters **3a** and **3b**. Acid-catalysed hydrolysis of the ester functions was performed in 3M hydrochloric acid to give the tetracarboxylic acid **4a** and the mixed acid **4b**. At last, in order to generate the structure **4c**, carboxylic functions were converted into phosphonic functions using H_3PO_3/PCl_3 according to the method of Krüger and Bauer (Krüger and Bauer, 1972). The other strategy described in scheme II required preparation of protected bis-carboxymethylated amino ethyl bromide. In view to convenience of deprotecting ethyl esters by acid-catalysed hydrolysis, *N,N-bis(*ethylacetate)-2-bromoethyl-amine was prepared according to the Williams and Rapoport's procedure (Williams and Rapoport, 1994) with minor modifications. N-alkylation of **2d** with the branching group in a mixed solvent system (CH_3CN/EtOH) at 70°C gave **3d** in 60% yield. Acid-catalysed hydrolysis of the ester functions as well as nitriles is the more convenient method (Ornstein et al., 1989), of hexacarboxylic acid **4d** preparation.

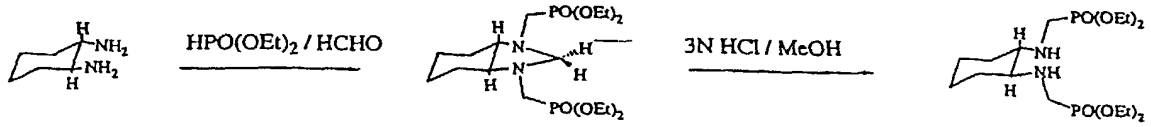


scheme I



scheme II

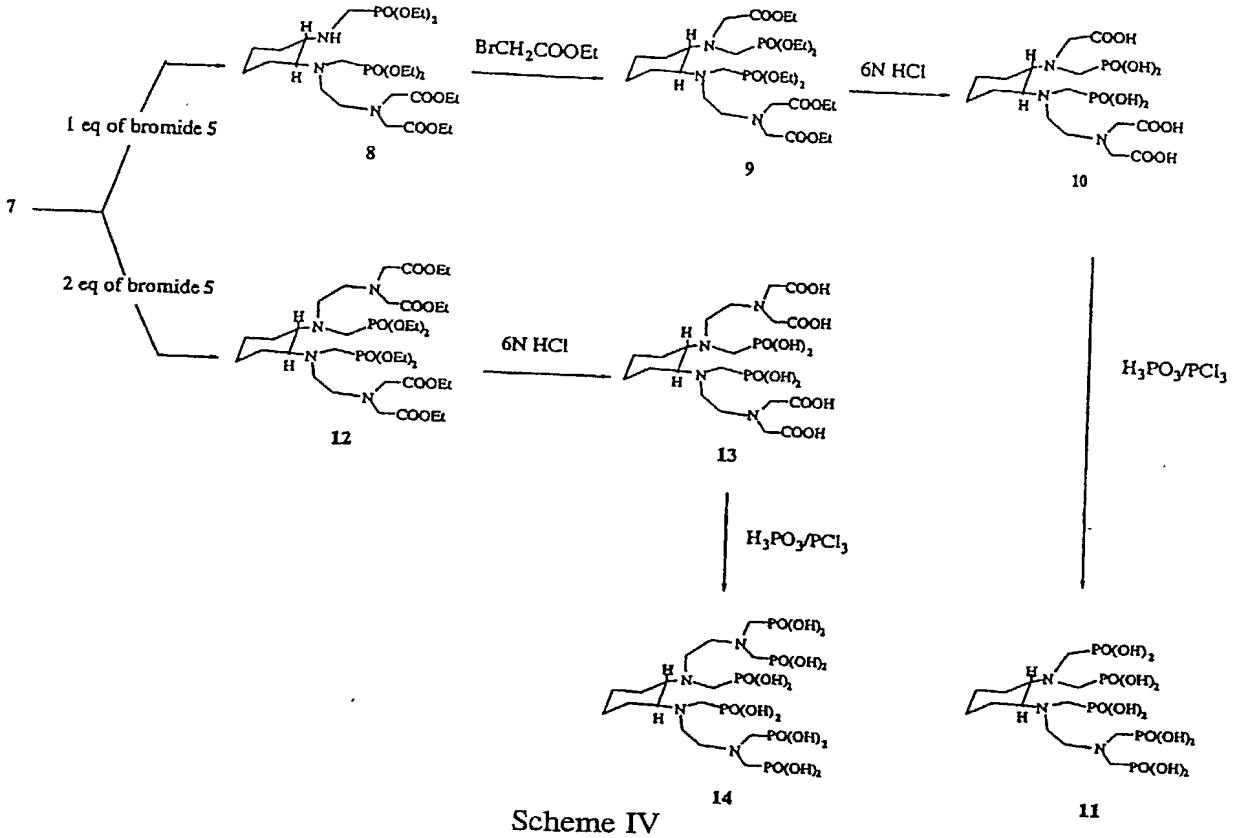
- In the case of certain diamines as *trans*-1,2-diaminocyclohexane **1**, the second route, as shown in schemes III, IV allowed the aminophosphonomethylation of amines protected by a methylene bridge between the two nitrogen atoms of **1**. This protecting group will subsequently provide for a different functionalization on the amine. The reaction of Kabachnick-field described and detailed by Baily and Burgada (Baily and Burgada, 1995), gave compound **6** which was prepared from paraformaldehyde and diethylphosphite in THF. **7** was obtained by removing the protecting group in acidic conditions.



1 **6** **7**

scheme III

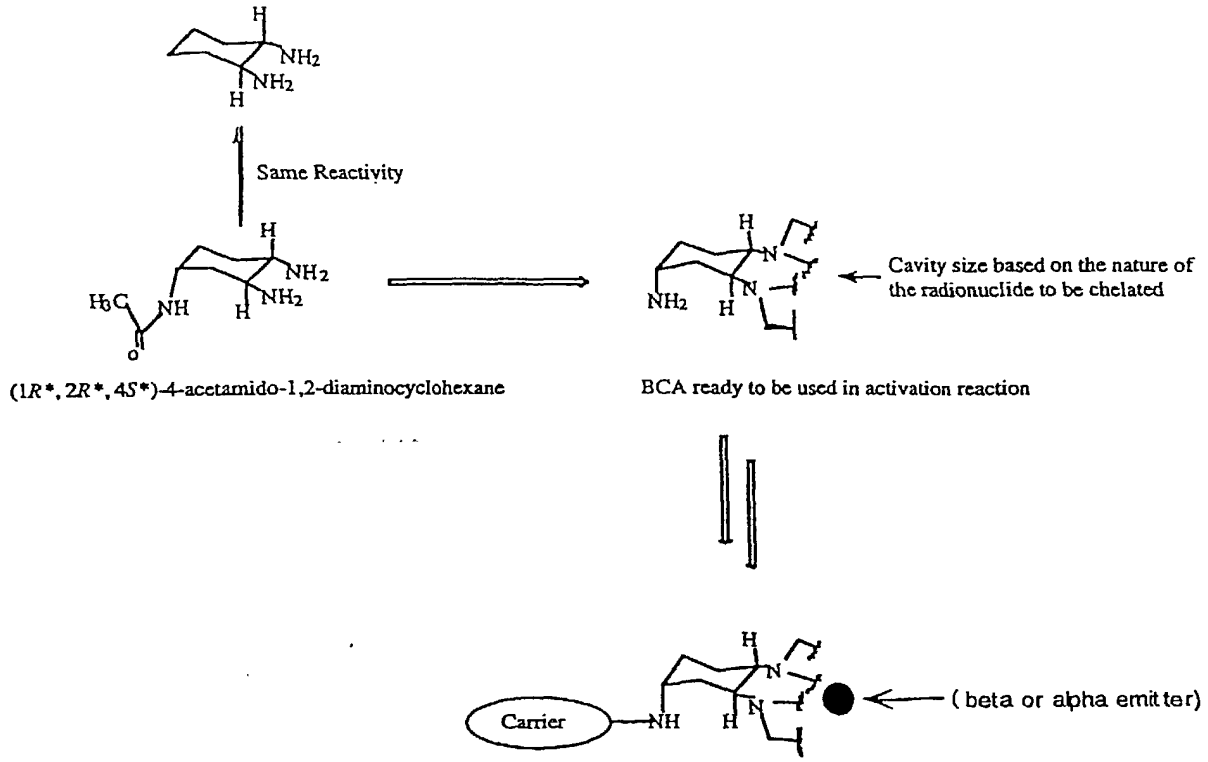
The monoalkylation or the dialkylation (see scheme IV), depending on the stoechiometry of the reaction gave respectively compounds **8** and **12**. The mixed acid **13** was obtained after hydrolysis of **12** in 6M hydrochloric acid and the hexaphosphonic acid **14** was prepared according to the method of Bauer and Kruger as described above. The synthesis of chelating agents **10** and **11** required an additional step which was the alkylation of **8** by ethyl bromoacetate to give the mixed ester **9**. Acid-catalysed hydrolysis gave **10** and **11** after reaction of conversion described all above.



Scheme IV

In conclusion, different non-functionalized ligands bearing aminophosphonate or aminocarboxylate chelate groups and mixed chelate groups were prepared and tested for their complexation properties with ^{153}Sm. The synthetic method described above was applied to the previously synthesized intermediate, the (1*R**, 2*R**, 4*S**)-4-acetamido-1,2-diaminocyclohexane, resulting in the synthesis of several polyaminocarboxylic acids, polyaminophosphonic acids and mixed semi-rigid functionalized ligands (BCA).

The different access routes to non-functionalized compounds described here were used without modifying the synthesis in order to obtain their functionalized homologues ready to be used in a coupling reaction as described in scheme V. We observed the influence of aminocarboxylic acid and aminophosphonic acid functions on the stability of the resulting complexes.



Scheme V

Experimental

General Procedures

All experiments were performed under nitrogen. Solvents were distilled prior to reactions. The primary chemicals used were commercial products (Sigma-Aldrich Company). Product purity and reaction progress were monitored on thin-layer chromatography (TLC) plates (60 F254, Merck), and liquid chromatography was carried out on a silica gel column (Merck 60,70-230 mesh). TLC revelation was performed under UV light (254 nm) or by iodine.

Nuclear Magnetic Resonance (MNR)

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 250 spectrometer (250 Mhz). Chemical shifts are reported in ppm to phosphoric acid as reference (85% H_3PO_4 in heavy water), positive values being downfield.

Chemical shifts (d) are reported in ppm. Coupling constant J is reported in Hertz (Hz).

Mass Spectrometry (MS)

MS spectra were recorded on a Mat Finnigan LCQ Ion Trap mass apparatus using the electrospray method in negative or positive mode.

Starting Material

The bisphosphonate **6** was prepared in our laboratory according the synthesis procedure of Baily and Burgada with minor modifications.

Synthesis and Specstroscopic data

***N,N'*-[(2-ethoxycarbonyl)eth-1-yl]-*trans*-cyclohexane-1,2-diamine 2a:** To freshly distilled *trans*-1,2-diaminocyclohexane 1 (1 ml, 8.33 mmol) in 50 ml of ethanol was added vinyl propionate (1.50 ml, 13.7 mmol) in one portion. After stirring 20h at room temperature, the reaction mixture was concentrated by rotary evaporation to yield a pale yellow oil (2.6 g, 8.32 mmol, 100%) witch was used directly in the next step. ^{1}H NMR ($CDCl_3$): d 1.22 (t, 12H), 1.67 (m, 2H), 1.82 (m, 2H), 2.06 (m, 2H+2H), 2.43 (t, 4H), 2.67 (dt, 2H), 2.98 (dt, 2H), 4.10 (q, 4H). ^{13}C NMR ($CDCl_3$): d 14.17, 24.31, 31.46, 35.34, 42.19, 60.23, 61.29, 172.69 . (M+H+): 315

***N,N'*-[(2-diethylphosphono)eth-1-yl]-*trans*-cyclohexane-1,2-diamine 2b:** To freshly distilled *trans*-1,2-diaminocyclohexane 1 (1 ml, 8.33 mmol) in 50 ml of ethanol was added diethyl vinyl phosphonate (2.80 ml, 18.21 mmol). The reaction mixture was allowed to stir at reflux during 15 hours. After removal the solvent under reduced pressure, the resulting oil was purified by column chromatography (silica gel, CH_2Cl_2-EtOH 1:1) to give 2.9 g of a limpid oil (6.65 mmol, 80%). ^{1}H NMR ($CDCl_3$): d 0.94 (m, 2H), 1.15 (m, 2H), 1.24 (t, 12H), 1.64 (m, 2H), 1.83-1.95 (m, 8H), 2.05 (m, 2H), 2.71 (dt, 2H), 2.97 (dt,

2H), 4.07 (dq, 8H). ^{13}C NMR ($CDCl_3$): d 16.37, 16.46, 25.42 (JC-P : 149 Hz), 28.20, 31.41, 40.47, 40.51, 61.29, 61.40, 61.50. (M+H+): 443

N,N'-[(2-cyano)eth-1-yl]-*trans*-cyclohexane-1,2-diamine **2d:** To freshly distilled *trans*-1,2-diaminocyclohexane 1 (1 ml, 8.33 mmol) in 50 ml of ethanol was added acrylonitryle (1.20 ml, 18.32 mmol). After stirring 20h at room temperature, the reaction mixture was concentrated by rotary evaporation to yield an pale yellow oil witch was purified by recristallisation in diethylether to give 1.40 g of a white solid (6.35 mmol, 78%): mp : 65°C

^1H NMR ($CDCl_3$): d 1.02 (m, 2H), 1.22 (m, 2H), 1.70-1.79 (m, 2H + 2H), 2.02-2.17 (m, 2H+2H), 2.49 (t, 4H), 2.80 (dt, 2H), 3.02 (dt, 2H). ^{13}C NMR ($CDCl_3$): d (M+H+): 221

N,N'-[(2-ethoxycarbonyl)eth-1-yl]-*N,N'*-(ethylacetate)-*trans*-cyclohexane-1,2-diamine **3a:** To a solution of 2a (1 g; 3,.18 mmol) in 50 ml of freshly distilled CH_3CN under nitrogen were added Na_2CO_3 (0.50 g; 3.01 mmol) and KI (g; mmol). After stirring for 1 hour at 60°C, $BrCH_2COOEt$ (1.80 ml; 7.15 mmol) was added dropwise. The reaction mixture was kept at this temperature over a period of 24 hours prior to cooling to room temperature, filtration and concentration under reduced pressure. The residue was taken up in $CHCl_3$ (200 ml) and washed with water. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give a yellow-brown oil. The crude product was purified by column chromatography (silica gel, CH_2Cl_2-EtOH 95:5). The fractions containing pure product were collected and dried to give an limpid oil (0.8 g; 1.56 mmol; 49 %). ^1H NMR ($CDCl_3$): d 1.10 (m; 4H), 1.26 (t, 12H), 1.91 (m, 2H), 2.00 (m, 2H), 2.51 (dt+m, 4H+2H(CH cycle)), 2.95 (dt, 4H), 3.39 (d, 4H), 4.12 (m, 8H). (M+H+): 515

N,N'-(ethylacetate)-*N,N'*-[(2-diethylphosphono)eth-1-yl]-*trans*-cyclohexane-1,2-diamine **3b:** The tetraester has been prepared as described above for compound from 1 g of compound 2b, Na_2CO_3 (0.70g, 6.60 mmol), KI (0.40, 2.40 mmol) and ethylbromoacetate (1.80 ml; 7.15 mmol). Purification by chromatography (SiO_2 (CH_2Cl_2-MeOH 99 : 1) gave 0.58 g of a pale yellow oil (0.94 mmol; 41%).^1H NMR ($CDCl_3$): d 1.11 (m, 4H), 1.25 (t, 6H), 1.29 (t, 12H), 1.70 (m, 2H), 1.85-2.10 (m, 4H+2H), 2.90 (t, 4H), 3.38 (d, 4H), 4.09 (m, 12H). ^{13}C NMR ($CDCl_3$): d 14.11, 16.31, 16.41, 25.63, 26.03 (JC-P : 135), 27.98, 44.86, 52.43, 60.26, 61.31, 61.41, 63.08, 172.46. (M+H+): 615

General procedure for preparation of corresponding acids: *trans*-cyclohexane-1,2-diamine-*N*,*N'*-acetic-*N*,*N'*-propionic acid 4a and *trans*-cyclohexane-1,2-diamine-*N*,*N'*-acetic-*N*,*N'*-ethylphosphonic acid 4b:

Compound 3a or 3b (1 g) was dissolved in 6N aqueous hydrochloric acid (12ml) and heated to reflux overnight. The refrigerant was removed, and the reaction mixture was kept at 70°C to dryness. An additional aqueous hydrochloric acid 6N (12 ml) was then added, and the solution was heated to dryness. the residue was taken up in MeOH and evaporated under reduced pressure. This step repeated twice gave the corresponding acid as an off-white solid, which was dried under vacuum and kept under nitrogen.

Compound 4a: ^{1}H NMR (D_2O): d 1.25-1.40 (m, 4H), 1.60-2.15 (m, 4H), 2.28 (m, 2H), 2.75 (t, 2H), 2.96 (t, 2H), 3.22 (m, 2H), 3.50-3.90 (m, 4H), 4.15 (s, 1H), 4.28 (s, 1H) ^{13}C NMR ($CDCl_3$): d 25.66, 26.22, 28.87, 31.20, 31.99, 47.25, 54.93, 66.92, 175.03, 176.46 (M-H+): 373

Compound 4b: ^{1}H NMR (D_2O): d 1.05-1.40 (m, 4H), 1.65-2.15 (m, 4H), 2.90-3.25 (m, 6H), 3.45-3.65(m, 2H), 3.70-3.95 (m, 2H). ^{13}C NMR ($CDCl_3$): d 25.66, 26.22, 28.87, 31.20, 31.99, 47.25, 54.93, 66.33, 176.73 (M-H+): 445

***trans*-cyclohexane-1,2-diamine-*N*,*N'*-ethylphosphonic-*N*,*N'*-methylphosphonic acid 4c:** A mixture of compound 4c (0.5 g; 1.12 mmol) and phosphorous acid (0.202 g; 2.46 mmol) in 10 ml of dry toluene was heated to 80°C and stirred for 30 min. PCl_3 (0.22 ml; 2.46 mmol) was then added dropwise, and the reaction mixture was kept at this temperature for 20 hours before being cooled to room temperature. The solvent was discarded and the residual product dissolved in a small volume of water. After filtration, the filtrate was evaporated to give a residue which was purified by precipitation in warm acetone and collected by filtration. The purification step was repeated twice to give 4c which was dried under vacuum and kept under nitrogen (0.460 g; 0.83 mmol; 74%). ^{1}H NMR (D_2O): d 1.15-1.65 (m, 4H), 1.75-2.10 (m, 2H), 2.15-2.40 (m, 6H), 3.00-3.60 (m, 10H). ^{13}C NMR ($CDCl_3$): d (M-H+): 517.

***N*,*N*-*Bis*(ethylacetate)-2-bromoethyl-amine 5:** Bromide 5 was synthesised in our laboratory according the synthesis procedure of Williams and Rapoport with minor modifications concerning the bis N-alkylated ethanolamine synthesis. To a 4°C solution of ethanolamine (6ml; 0.1 mol) in 100 ml of dried acetonitrile was added dropwise ethylbromoacetate (7.4 ml; 66 mmol) over a period of 20

min during which time a large quantity of precipitate formed. The mixture was allowed to stir for 2 hours at this temperature. The white solid was removed by filtration and washed with a small quantity of acetonitrile. The filtrate was concentrated under reduced pressure. The resulting liquid was taken up in $CHCl_3$ (100mL) and washed with water. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give a liquid which was used directly in the next step (5.9 g; 25.32 mmol; 77%). The dialkylated ethanolamine and Ph_3P (7.72 g; 27.9 mmol) were dissolved in CH_2Cl_2 (100mL). The mixture was cooled in an ice bath and vigorously stirred while NBS (4.96 g; 27.9 mmol) was added in small portions. After the solution was stirred at 0°C for two hours, evaporation of the solvent gave a semisolid which was tritured with ether and the resulting solid was separated by filtration. the filtrate was evaporated to give an oil which was purified by column chromatography (silica gel, CH_3Cl). (6.14 g; 20.7 mmol; 62% overall) ^1H NMR ($CDCl_3$): d 1.26 (m, 6H), 3.15 (t, 2H, J = 7.75 Hz), 3.44 (t, 2H, J = 7.75 Hz), 3.59 (s, 4H), 4.15 (q, 4H). ^{13}C NMR ($CDCl_3$): d (M+H+): 297

***N,N'*-[(2-cyano)eth-1-yl]-N,N'-[*N'',N''*-*bis*-(ethylacetate-2-aminoethyl)]-*trans*-cyclohexane-1,2-diamine 3d:** To a solution of compound 2d (1 g; 4.54 mmol) and bromide 5 (3 g; 10.13 mmol) in a mixed solvent system (CH_3CN-EtOH, 1:1) was added Na_2CO_3 (1.4 g; 13.20 mmol) and KI (0.75 g; 4.54 mmol). After stirring for 2 days at 70°C, the reaction mixture was filtered and concentrated under reduced pressure. The residue was taken up in $CHCl_3$ (200 ml) and washed with water. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give a yellow-brown oil. The crude product was purified by column chromatography (silica gel; CH_2Cl_2-EtOH 98:2). The fractions containing pure product were collected and dried to give an limpid oil (1.09 g; 1.68 mmol; 37%).^1H NMR ($CDCl_3$): d 1.13 (m, 4H), 1.27 (t, 12H), 1.73 (m, 2H), 1.86 (m, 2H), 2.30-2.85 (m, 4H+2H), 2.95 (m, 2H), 3.55 (s, 8H), 4.17 (m, 12H). ^{13}C NMR ($CDCl_3$): d 14.25, 18.63, 25.79, 27.24, 47.35, 48.98, 53.78, 55.43, 60.54, 62.64, 119.49, 171.11. (M+H+): 652

***trans*-cyclohexane-1,2-diamine-*N,N'*-propionic-*N,N'*-[*N'',N''*-*bis*-(2-aminoethyl)]-tetra-acetic acid 4d:** this hexaacid has been prepared as described above for compounds 4a & 4b from 1 g of the ester 3d and two volumes of 20 ml HCl (6N). ^1H NMR (D_2O): 1.20-2.00 (m, 10 H), 2.30 (m, 2H), 2.40-3.00 (m, 4H), 3.10-3.95 (m, 14 H), 4.20 (m, 4H) d ^{13}C NMR (D_2O) : d 26.89, 30.49, 40.99, 52.00, 55.18, 55.47, 58.97, 165.50, 170.38. (M-H+): 547

N,N'-(diethylphosphono-methyl)-*trans*-cyclohexane-1,2-diamine 7: was synthesised in our laboratory according the synthesis procedure of Baily and Burgada with minor modifications. Freshly distilled *trans*-1,2-diaminocyclohexane 1 (3.6 ml, 30 mmol) and diethyl phosphite (7.24 ml, 60 mmol) were dissolved in THF (40ml). The mixture was stirred at reflux and paraformaldehyde (2.8 g, 93 mmol) was added over a 30-min period and the reaction mixture was stirred at reflux for 4 hours. The solvent was evaporated to afford a residue which was taken up in $CHCl_3$. The organic layer was washed with brine (2*100 ml), dried and evaporated to leave a crude oil. A purification by column chromatography (silica gel, CH_2Cl_2-EtOH 96:4) gave 6 (9.2 g, 21.60 mmol, 72%). 6 was then dissolved in MeOH (40 ml) and 35% HCl (15 ml) was added. The mixture was stirred at 50°C overnight. MeOH was removed. the aqueous layer was adjusted to 50 ml with H_2O and then neutralized by $HNaCO_3$. bisphosphonate 7 was extracted by $CHCl_3$. Organic layers were collected, dried and evaporated to give 5.6 g of bisphosphonate 7 (13.52 mmol, 62%, 45% overall). ^1H NMR ($CDCl_3$): 1.10 (m, 2 H), 1.27 (t, 12H), 1.48 (m, 2H), 1.76 (m, 2 H), 2.13 (m, 2H), 2.98 (m, 2H), 3.10 (t, 2H), 3.35 (t, 2H), 4.12 (m, 8H). ^{13}C NMR ($CDCl_3$): d: 16.36, 16.39, 16.45, 16.38, 24,01, 28.23, 39.67 (JP-C = 156 Hz), 60.74, 60.89, 63.16, 63.23, 63.26, 63.33. (M+H+): 415

N,N'-(diethylphosphono-methyl)-*N*-[*N'',N''*-*bis*-(ethylacetate-2-aminoethyl)]-*trans*-cyclohexane-1,2-diamine 8: The mixed ester has been prepared in a mixed solvent system (CH_3CN-H_2O, 1:1) as described above for compound 3d from bisphosphonate 6 (1 g, 2.41 mmol) , Na_2HPO_4 (0.5 g; 3.50 mmol) and 1 equivalent of bromide 5 (0.71 g; 2.41 mmol). After stirring for 2 days at 70°C, the reaction mixture was filtered and concentrated under reduced pressure.. The residue was taken up in $CHCl_3$ (200 ml) and washed with water. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give a yellow-brown oil witch was used directly in the next step. ^1H NMR: 1.00-1.20 (m, 4 H), 1.25 (t, 6H), 1.31 (t,12H), 1.72 (m, 2H), 2.04 (m, 2H), 2.23 (m, 1 H), 2.70-2.95 (m, 6H), 3.12 (m, 2H), 3.65 (s, 4H), 4.15 (m, 18H).

N,N'-(diethylphosphono-methyl)-*N'*-(ethylacetate)-*N*-[*N'',N''*-*bis*-(ethylacetate-2-aminoethyl)]-*trans*-cyclohexane-1,2-diamine 9: The tetraester is prepared as described above for compounds 3a & 3b from compound 8,

Na_2CO_3, KI and ethylbromoacetate (1 equivalent). Purification by chromatography (SiO_2) gave a oil.

N,N'-(diethylphosphono-methyl)-*N,N'*-[*N'',N''*-*bis*-(ethylacetate-2-aminoethyl)]-*trans*-cyclohexane-1,2-diamine **12**: The mixed ester is prepared as described above for compound 3d from bisphosphonate 7 (1g, 2.41 mmol), Na_2HPO_4 (1 g, 7.04 mmol) and 2 equivalents of bromide 5 1.80 g, 6.08 mmol). Purification by chromatography (SiO_2 (CH_2Cl_2-MeOH 95 : 5) gave a pale yellow oil (0.59 g, 0.70 mmol, 29%). ^1H NMR: 1.16 (m, 4H), 1.25 (t, 12H), 1.33 (t, 12H), 1.69 (m, 2H), 1.86 (m, 2H + 2H), 2.70-3.50 (m, 8H + 2H + 2H), 3.57 (s, 8H), 4.12 (m, 16H). (M+H+): 847

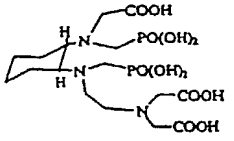
***trans*-cyclohexane-1,2-diamine-*N,N'*-methylphosphonic-*N*-*acetic*-,*N'*-[*N'',N''*-*bis*-(2-aminoethyl)]-tetra-acetic acid 10 & *trans*-cyclohexane-1,2-diamine-*N,N'*-methylphosphonic-*N,N'*-[*N'',N''*-*bis*-(2-aminoethyl)]-tetra-acetic acid 13** : Those compounds are prepared as described above for compound 4a, 4b and 4d.

Compound 13: ^1H NMR (D_2O): 1.26 (m, 2 H), 1.45 (m, 2H), 1.75-2.00 (m, 4H), 2.80 (m, 2H), 3.00-3.70 (m, 12H), 4.10 (br s, 8H). (M-H+): 619

***trans*-cyclohexane-1,2-diamine-*N,N,N'*-methylphosphonic-*N'*-[*N'',N''*-*bis*-(2-aminoethyl)]-di-methylphosphonic acid 11 & *trans*-cyclohexane-1,2-diamine-*N,N'*-methylphosphonic-*N,N'*-[*N'',N''*-*bis*-(2-aminoethyl)]-tetra-methylphosphonic acid 14 :** Those compounds are prepared as described above for compound 4c.

153Sm complexation studies

Complexation studies with Samarium 153 were performed on the two following chelating agents 10 (AL 247) and 13 (AL 245)



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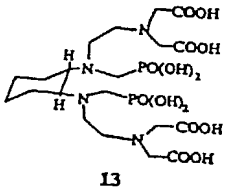

13

Radiochemistry purity was measured on ITLC-SG chromatographic profiles. Radioactivity was quantified using a Phosphorimager 445SI apparatus.

Samarium 153 was furnished under $^{153}SmCl_3$ form in HCl 0,04N with a 5,2 GBq/ml volumic activity and a 40 GBq/mg specific activity.

They were tested for their complexation properties by using an excess of 10 to 50 equivalents of chelating agent.

Competition studies were performed against EDTMP according to the following method :

- first step : 50 equivalents of one of the chelating agents and a fixed amount of 153Sm were incubated at 37°C during 3 hours in order to form the 153Sm-CA complex (CA = Chelating Agent),

- second step : 50 equivalents of EDTMP were added to the previous solution and kept 3h at 37°C in order to measure the decomplexation. Another measure was performed 72h after to ensure a complete decomplexation possibility.

Results :

	% of non decomplexed 153Sm-CA	
chelating agent	after 3h	after 72h
10 (AL 247)	100	67
13 (AL 245)	100	100

AL 247 and AL 245 present very good complexation properties for 153Sm and in any cases better than EDTMP

Furthermore, stability was performed on AL 245 in human serum media at 37°C at different time and showed no loss of 153Sm from AL 245 at either 24, 48, 72 and 96h.

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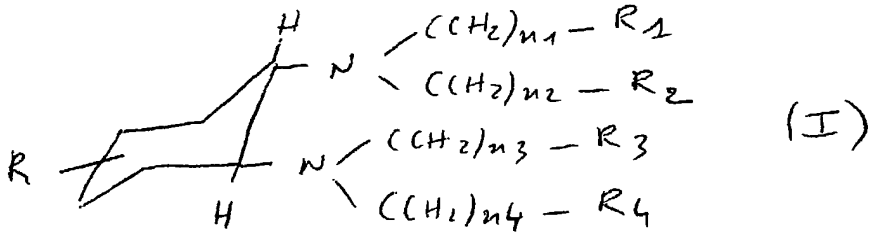
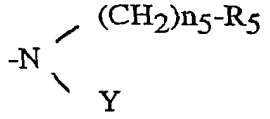
CLAIMS

1. Compounds of the following formula (I) :



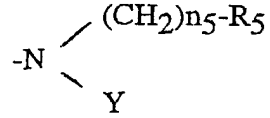
(I)

in which :

- n_1, n_2, n_3 and n_4, independently from each other, represent an integer from 1 to 5, preferably from 1 to 3,
- R_1, R_2, R_3 and R_4, independently from each other, represent :
  - . -COOH,
  - . $-PO(OH)_2$,
  - . $-N\begin{  $

wherein n_5 represents an integer from 1 to 5, preferably from 1 to 3, R_5 represents –COOH or $-PO(OH)_2$, and Y represents H or a group $-(CH2)n_6-R_6$ in which n_6 represents an integer from 1 to 5, preferably from 1 to 3, and R_6 represents –COOH or $-PO(OH)_2$,

provided that at least one of R_1, R_2, R_3 or R_4 represents a group

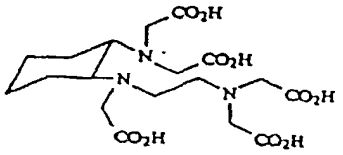


such as defined above,

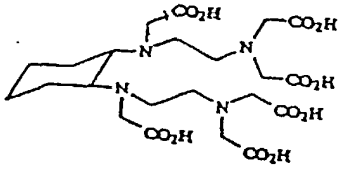
- R represents :
  - . H, or $-NHCOCH_3$, or
  - . a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, which are able to bind specifically with epitopes located at the surface of the cells of the organism, or to chemical or biological compounds located at the surface of a solid carrier, or

. a group carrying a function linked, if necessary via a binding site, to molecules as defined above,

the two following compounds, CDTPA and CTTHA, being excluded :



CDTPA

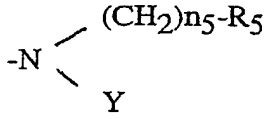


CTTHA

2. Compounds according to claim 1, characterized in that :

- when R_1, R_2, R_3 or R_4 represents -COOH or -PO(OH)2, then n_1, n_2, n_3 or n_4 represents 1 respectively,

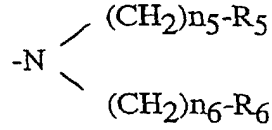
- when R_1, R_2, R_3 or R_4 represents a group



then n_1, n_2, n_3 or n_4 represents 2 or 3 respectively, and preferably 2,

- n_5, and optionally n_6, represents 1.

3. Compounds according to claims 1 or 2, characterized in that at least one, and more preferably two of R_1, R_2, R_3 and R_4, represent a group



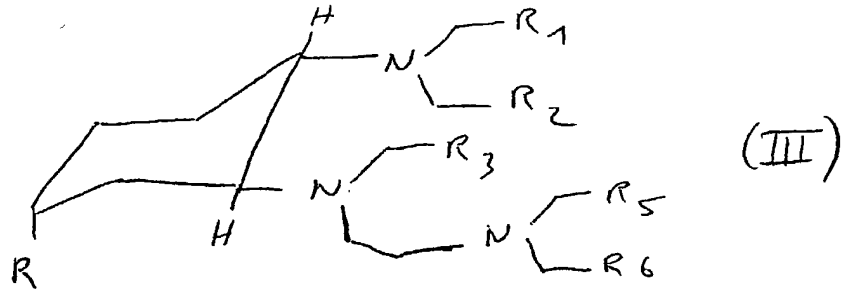
wherein n_5, n_6, R_5 and R_6 are defined in claims 1 or2.

4. Compounds according to anyone of claims 1 to 3, characterized in that R represents a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined in

claim 1, and in particular R represents a group chosen among all the coupling functions for vector or solid support binding.

5. Compounds according to anyone of claims 1 to 3, characterized in that R represents a group carrying a function linked, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined in claim 1.

6. Compounds according to anyone of claims 1 to 5 of the following formula (III) :



(III)

in which R_1, R_2, R_3, R_5 and R_6 independently from each other represent -COOH or -$PO(OH)_2$, and R is a group as defined in claims 2 to 5.

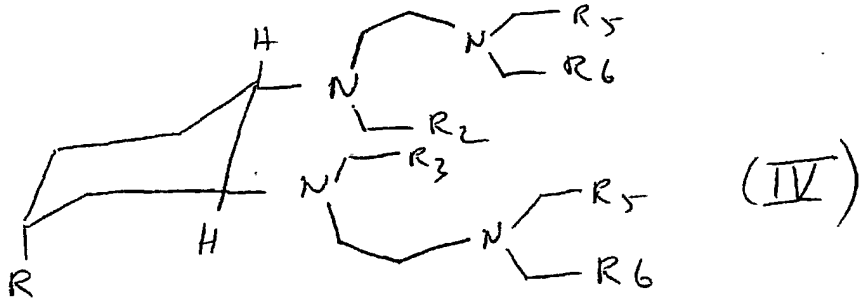
7. Compounds according to claim 6, of formula (III) wherein :

. $R_1 = R_5 = R_6 = COOH$ and R_2, $= R_3 = PO(HO)_2$, or

. $R_1 = R_2 = R_3 = R_5 = R_6 = COOH$, or

. $R_1 = R_2 = R_3 = R_5 = R_6 = PO(OH)_2$.

8. Compounds according to anyone of claims 1 to 5, of the following formula (IV) :



(IV)

wherein R_2, R_5 and R_6, independently form each other, represent -COOH or -$PO(OH)_2$, and R is a group as defined in claims 2 to 5.

9. Compounds according to claim 8 of formula (IV) wherein :
. $R_2 = R_3 = PO(OH)_2$, and $R_5 = R_6 = COOH$, or
. $R_2 = R_3 = R_5 = R_6 = COOH$, or
. $R_2 = R_3 = R_5 = R_6 = PO(OH)_2$.

10. Complexes between a compound according to anyone of claims 1 to 9, and a radioactive element.

11. Complexes according to claim 10, characterized in that said radioelements are α or β emitter radiometals.

12. Complexes according to claim 11, characterized in that the compound is chosen among those defined in anyone of claims 5 to 9, and more particularly among those compounds wherein the group R comprises :
- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,
- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound, in a first step of the treatment, to epitopes on the surface of specific cells in the organism,.
- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

13. Use of a complex according to claims 11 or 12, for the manufacture of a medicament for radioimmunotherapy, such as for the treatment of cancers, and more particularly for the treatment against metastase proliferation.

14. Pharmaceutical compositions characterized in that they comprise an effective amount of a complex according to claims 11 or 12, in association with a suitable pharmaceutical carrier.

15. Complexes according to claim 10, characterized in that the radioelements are γ emitter radiometals.

16. Complexes according to claim 15, characterized in that the compound is chosen among those defined in anyone of claims 5 to 9, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,

- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound (in a first step of the method of diagnosis) to epitopes on the surface of specific cells in the organism,

- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

17. Use of a complex according to claims 15 or 16, for carrying out diagnosis methods such as radioimmunoscintigraphy.

18. Use of a compound of formula (I) as defined in claim 1 to 9, included compounds CDTPA and CTTHA, for :

- the manufacture of a medicament useful as antalgic, or for the treatment of pathologies where ionic imbalances occur, or against the formation of stones in the organism,

- carrying out a process for the detoxication of polluted medium, such as liquid phases polluted by bivalent or trivalent metals radioactives or not,

- carrying out a process for the radionuclides purification, said compound being bound to a solid phase,

- carrying out a bone scintigraphy, in particular in the frame of the diagnosis of osteoarticular pathology, particularly in bone cancer extension balance.

ABSTRACT OF THE DISCLOSURE

The invention relates to compounds of formula (I): in which : n_1, n_2, n_3 and n_4, represent an integer from 1 to 5, R_1, R_2, R_3, and R_4, independently form each other, represent -COOH, $-PO(OH)_2$ at least R_1, R_2, R_3, or R_4 represents a group (II), wherein n_5 represents an integer from 1 to 5, R_5 represents -COOH or $-PO(OH)_2$, and Y represents H or a group $-(CH2)n_6-R_6$ in which n_6 represents an integer from 1 to 5, and R_6 represents -COOH or $-PO(OH)_2$, R represents H, or a group carrying a function linked to molecules able to bind with epitopes at the surface of cells. The invention also relates to the processes of preparation of the compounds, and to their use in pharmaceutical compositions and in diagnosis methods.

Ref. USB 98 BA INS SAM

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CHELATING AGENTS FOR RADIOIMMUNOTHERAPY

the specification of which: *(check one)*

REGULAR OR DESIGN APPLICATION

[ ] is attached hereto.

[ ] was filed on as application Serial No. and was amended on (if applicable).

PCT FILED APPLICATION ENTERING NATIONAL STAGE

[X] was described and claimed in International application PCT/EP99/08031 filed on 22 October 1999 and as amended on (if any).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

PRIORITY CLAIM

I hereby claim foreign priority benefits under 35 USC 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

PRIOR FOREIGN APPLICATION(S)

Country	Application Number	Date of Filing (day, month, year)	Priority Claimed
Europe	**98402648.4**	**23 October 1998**	**yes**

(Complete this part only if this is a continuing application.)

I hereby claim the benefit under 35 USC 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 USC 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.) (Filing Date) (Status--patented, pending, abandoned)

POWER OF ATTORNEY

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from **Grosset-Fournier & Demachy** as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

As a named inventor, I hereby appoint the registered patent attorneys represented by Customer No. **000466** to prosecute this application and transact all business in the Patent and Trademark Office connected therewith, including: **Robert J. PATCH, Reg. No. 17,355, Andrew J. PATCH, Reg. No. ~~32,925,~~ Robert F. HARGEST, Reg. No. ~~25,590,~~ Benoît CASTEL, Reg. No. ~~35,041~~, Eric JENSEN, Reg. No. 37,855, Thomas W. PERKINS, Reg. No. 33,027, and Roland E. LONG, Jr., Reg. No. 41,949,**

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 09/830188	INTERNATIONAL APPLICATION NO. PCT/EP99/08031	ATTORNEY'S DOCKET NO. 98 BA INS SAM

17. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

Neither international preliminary examination fee (37 CFR1.482) nor international search fee (37 CFR1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO . . . . . $ 1,000.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO . . . . . $ 860.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO . . . . . $ 710.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) . . . . . $ 690.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) . . . . . $ 100.00

	CALCULATIONS PTO USE ONLY
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00
Surcharge of $130.00 for furnishing the oath or declaration later than 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	18 - 20 =	0	X $18.00	$
Independent claims	1 - 3 =	0	X $80.00	$
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			+ $270.00	$

TOTAL OF ABOVE CALCULATIONS =	$ 990.00
Reduction of ½ for filing by small entity, if applicable. Applicant claims Small Entity Status under 37 CFR 1.27. +	$
SUBTOTAL =	$ 990.00
Processing fee of $130 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR1.49(f)).	$
TOTAL NATIONAL FEE =	$ 990.00
Fee for recording the enclosed assignment (37 CFR1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +	$
TOTAL FEES ENCLOSED =	$ 900.00
Amount to be refunded:	
charged:	

a. [X] A check in the amount of $ **990.00** to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. **25-0120** in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required by 37 CFR 1.16 and 1.17, or credit any overpayment to Deposit Account No. **25-0120**. A duplicate copy of this sheet is enclosed.

SEND ALL CORRESPONDENCE TO:

Customer No. 000466
YOUNG & THOMPSON
745 South 23rd Street
2nd Floor
Arlington, VA 22202
(703) 521-2297 facsimile (703) 685-0573

April 23, 2001

By Benoît Castel

Benoît Castel
Attorney for Applicants
Registration No. 35,041